APPENDIX C: PREPARATION OF YIELD AND PRODUCT GELS

1 EQUIPMENT

1.1 Graduated Cylinder

1.2 Erlenmeyer or round bottle flask

1.3 Microwave oven

1.4 Gel beds, Lifecodes (12.0 cm x 16.0 cm) or BRL (11.0 cm x 20.0 cm)

1.5 Gel lane combs, Lifecodes (25 lane) or BRL (20 lane)

1.6 Leveler

1.7 Pipette - 10 or 20 µL

1.8 Orbital platform shaker

1.9 Water bath or oven, 65°C

1.10 Balance, top loading

2 MATERIALS

2.1 Tape

2.2 Large bore transfer pipette

2.3 Weighing paper

2.4 Sterile tips for pipettes

2.5 Plastic wrap

2.6 Ziploc bag

3 REAGENTS

3.1 1X TAE

3.2 0.5X TBE

3.3 Nusieve Agarose

3.4 DNA Typing Grade Agarose

3.5 Ethidium bromide

3.6 Type I Water

4 PROCEDURE

NOTE: The Yield Gel procedure uses 1X TAE and 1.0% DNA Typing Grade Agarose and the Product Gel procedure uses 0.5X TBE and 3.0% NuSieve Agarose.

4.1 Calculate the amount of agarose and 1X TAE or 0.5X TBE needed. Use 100 mL of 1X TAE or 0.5X TBE for a 12.0 cm x 16.0 cm gel and 115 mL for a 11.0 cm x 20.0 cm gel. Multiply the desired percentage of the gel by the volume of TAE or TBE to obtain the correct amount of agarose required.

EXAMPLE: 1.0% 16.0 cm gel

(1.0%)(100 mL) ~ 1.0 g of agarose

4.2 Weigh the agarose and pour it into an Erlenmeyer flask. Measure the 1X TAE or 0.5X TBE in a graduated cylinder and pour into the Erlenmeyer flask. The capacity of the flask should be at least 2 times the volume of the buffer to avoid boiling over in the microwave oven. Loosely cover the flask top with plastic wrap and swirl. Heat in a microwave oven to dissolve. Heating time will depend upon the volume and number of flasks, as well as the characteristics of the microwave oven.

EXAMPLE: For a 100 mL volume: Heat 1-1.5 minutes in a microwave oven to reach boiling. Remove the flask from the heat source, determine the volume and bring back to original volume with Type I Water. Place on an orbital shaker, and rotate for 5 minutes at 90 rpm. If necessary, return the flask to the heat source and heat for 30-45 seconds to completely dissolve the agarose.

4.3 If the gel is to be poured immediately, remove the flask from the heat source, place it on the orbital shaker and rotate for 10 minutes at 90 rpm (until agarose cools). If the gel is not to be poured immediately, molten agarose may be kept in a 65°C water bath or oven until it is poured. Reconfirm the original volume at this time. When the flask is cool enough to handle (approximately 15 minutes at room temperature or 30 minutes at 65°C), the agarose can be poured into prepared gel beds. Make sure it is not too hot as it will melt the adhesive tape and cause leakage.

NOTE: Following boiling and rotating for 5 minutes at 90 rpm, the flask may also be cooled down by swirling under running tap water.

4.4 Prepare gel beds by taping the ends closed, being careful to seal edges. Level the gel bed.

4.5 For yield gel and/or product gel, 10 µL of 5 mg/mL ethidium bromide stock solution per 100 mL of agarose may be added. If ethidium bromide is not added to the agarose, the gel may be stained with the same concentration after electrophoresis is complete.

CAUTION: ETHIDIUM BROMIDE IS A MUTAGEN. AVOID DIRECT CONTACT. ALWAYS WEAR GLOVES WHEN HANDLING ETHIDIUM BROMIDE AND ETHIDIUM BROMIDE GELS.

4.6 Pour molten agarose into center of gel bed. Remove any bubbles with the tip of a transfer pipette. Place the comb in position. Allow the gel to solidify for approximately 30 minutes at room temperature.

4.7 Place the gel, while still on the gel bed with the comb in place, into a plastic Ziploc bag along with a moistened Kimwipe and refrigerate for a minimum of 15 additional minutes prior to removing the comb.

4.8 If the gel will not be used immediately, carefully remove the comb and store the gel in the Ziploc bag with a moistened Kimwipe in a refrigerator until used.

♦**END**